Chapter 5

5.1 Introduction

Chemotherapy is one of the most suitable approaches for cancer treatment, where single or combination of drugs are used to be delivered at the active site. However, the therapeutic efficacy of anti-cancerous drug is poor due to their limited penetration in tumor tissues along with adverse side effects caused to normal healthy cells.[177] Since the drug concentration at the tumor site is restricted, therefore, more doses are required which lead to well known toxicity and higher possibility of drug resistance and metastasis.[178] Thus, control delivery of anti cancerous drugs to specific tumor cells is of academic as well as major healthcare challenges. To overcome the short comings, development of drug carriers to reach the drug molecule into the affected cells with minimum dose with better efficiency has a long term goal of the reasearchers.[179, 180] So far, polymer based carriers are known that preferentially accumulate in tumor tissues and then into tumor cells followed by releasing the encapsulated drug for a prolong time, thereby, reduces the adverse side effects of drug through improved therapeutic efficiency.[181] Chemical modification by grafting the synthetic polymers onto natural polysaccharide backbone offers the combined advantages for wide range of applications as per the requirements. For example, modification of cellulose with synthetic polymers are used for gene delivery,[182] drug carrier[183] and smart hydrogels.[184] Graft copolymers draw certain advantages over block copolymer in terms of synthetic route and having large number of reaction sites along the backbone peripheries as opposed to block copolymer where the reaction proceeds usually at chain ends.[185] The branching architecture has remarkable influence on control

drug release as compared to traditional linear polymer carriers. The degree of branching in polymer backbone influences the drug release, entrapment efficiency and cell toxicity.[186] Cyclodextrin (CD) is widely used as host molecules since they are non toxic, water soluble, readily functionalized due to multiple hydroxyl groups and has better complexation ability with variety of hydrophobic molecules to regulate the hydrophilic-hydrophobic balance. CD based polymer networks having multiple CD units threaded or bound on a polymeric three dimensional structures are able to hold guest molecules, that are too large to be accommodated inside the single CD cavity, due to network arrangement of CD units in a polymeric frame.[187, 188] The strategy of sustained delivery is not enough as most of the cases they do not exhibit site specific or targeted delivery. Hydrogels are versatile and most promising material for drug delivery[189, 190] due to its high water content and sufficient strength of hydrogels which can mimic extracellular matrices and can broadly be used in tissue engineering.[191] Hydrogels, designed using a natural polymer have received a great deal of attention due to its biocompatibility and low toxicity.[191-196] Numerous hydroxyl groups in CD are the active sites that can form chemical linkages with diisocyanate forming a network structure with polyurethane chains.[197] Various polymeric hydrogels are reported for anti-cancerous drug delivery mostly in terms of slight tumor growth suppression.[198-200]

Here, with an aim to completely heal the tumor, especially melanoma, we have designed a different architecture of cyclodextrin up to third generation using a linear aliphatic diisocyanate as chemical connector to create a superstructure (3G) followed by grafting with suitable length of polyurethane chain in the periphery of the superstructure to make a hydrophilic core and hydrophobic shell for better control release of guest molecule like

drug (paclitaxel) from the overall superstructure. The drug loaded superstructure is inoculated in methyl cellulose (MC) to convert the whole system as injectable hydrogel for its subcutaneous administration to artificially induced melanoma bearing mice. The modified drug loaded hydrogel *i.e.* 3G-g-PU-D in methyl cellulose slowly release the drug specifically at the tumor site and kill the melanocytes by releasing the paclitaxel drug in a control fashion for longer time for realising its full potential. To validate this system, we studied the formation of a superstructure, their sustained delivery and efficacy in animal model for complete healing of tumor.

5.2 Results and discussion

5.2.1 Spectroscopic characterization

The sequence of reaction scheme to synthesize various generations of CD is done by control chemical reaction between hydroxyl group of CD and diisocyanates yielding branched structures, as shown in ***scheme 2.3*** in experimental section which are confirmed through ^{1}H NMR and FTIR studies. The presence of the peak at chemical shift of δ~8 ppm in the spectra of all generations is attributed to urethane proton formed by the reaction of CD and hexamethylene diisocyanate (HMDI) and its intensity increases with increasing urethane linkages in higher generation (***Figure 5.1a***). The chemical tagging of prepolymer onto 3G is understood from the appearance of peak at δ~4 ppm due to proton of HMDI in the peripheral polyurethane chains along with another new intense peak at δ~1.5 ppm, assigned to methylene proton of PTMG. The broad FTIR peak at 3345 cm^{-1} in pure CD gets narrowed as well as shifted to higher wavenumber to 3461, 3463 and 3483 cm^{-1} in 2G, 3G and 3G-g-PU, respectively, due to the conversion of -OH groups into urethane linkages (**Figure 5.1b**).

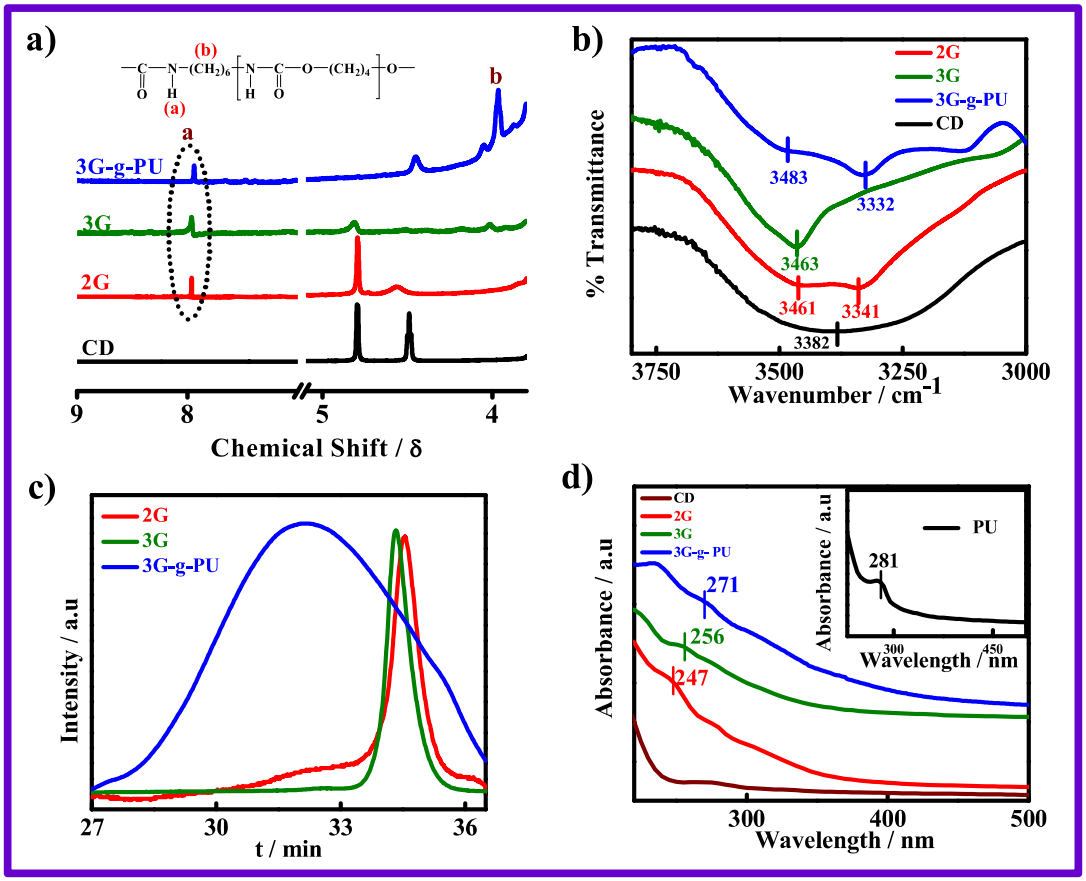

Figure 5.1: *a) ^{1}H NMR Spectra of CD, and their indicated generations showing new peak position due to grafting marked by 'a' and 'b'. b) FTIR spectra of pure CD and its generations and superstructure as indicated showing shifting of peak position arising from interaction; c) Gel permeation chromatograms of generations showing lower elution time for 3G-g-PU w.r.t 2G/3G; and d) UV-Vis spectra of pure CD, its generation and superstructure. Vertical lines indicate the peak position for n→π* transition of carbonyl peak. Inset figure show the UV-vis pattern of pure polyurethane.*

Both inter- and intra-molecular hydrogen bonding persists in less branched 2G system, as reflected from the appearance of two separate peaks at 3461 and 3341 cm^{-1} while prominent intramolecular hydrogen bonding (one peak) is observed in higher branched (3G) system. On contrary, wrapping of prepolymer on 3G (3G-g-PU) exhibits considerable peak shifting at 3483 cm^{-1} due to intramolecular hydrogen bonding along with inter-molecular hydrogen bonding peak at 3332 cm^{-1} and thereby form a constraint

superstructure. Appearance of the absorption peaks at 1720 and 1580 cm^{-1} clearly indicates the presence of urethane carbonyl and NH moiety in the superstructure.[201] Low elution time peak (high molar mass) in gel permeation chromatogram is evident for 3G-g-PU vis-à-vis narrow and high elution time peak (low molar mass) for 2G and 3G clearly indicate the grafting of polyurethane chain in the periphery of 3G structure (***Figure 5.1c***). Interactions between polymer chains are visualized through UV-Vis spectroscopy and systematic red shift of $n\rightarrow\pi^*$ transition of carbonyl peak is observed at 247, 256 and 271 nm for 2G, 3G and 3G-g-PU, respectively, indicate strongly interactive system with higher generation followed by peripheral grafting of polyurethane as opposed to 281 nm peak in pure PU of the similar electronic transition (***Figure 5.1d***). This is to mention that pure CD does not show this band as expected while hydrogen bonding and dipolar interactions in various generations and superstructures are responsible for red shift of the transition.

5.2.2 Structure and Morphology

As a result of strongly interactive system, highly crystalline nature of pure CD is significantly reduced in higher generation and superstructure as evident from the disappearance of most XRD peaks while better interaction enhances the blob/agglomerate size (0.3, 0.5 and 0.8 nm for 2G, 3G and 3G-g-PU, respectively) in higher generation and superstructure as evident from the Debye-Buche fitting of the small angle neutron scattering data of different generations and superstructure (***Figure 5.2a,b)***. Layered assembly followed by peripheral grafting of polyurethane increase the size of the blob and ultimately its constraint structure facilitate holding the embedded guest molecules like drug. The efficacy of grafting for control release is well illustatred from the particle size as shown through TEM images of native CD and 3G-g-PU indicating reduced particle size

after grafting of PU on 3G architecture (***Figure 5.2c)***. Large clusters are found in CD due to its hydrophilic nature and strong intermolecular hydrogen bonding present between the chains which gets disrupted upon grafting with 3G leading to smaller particles of size of 50 nm against 170 nm in pure CD.

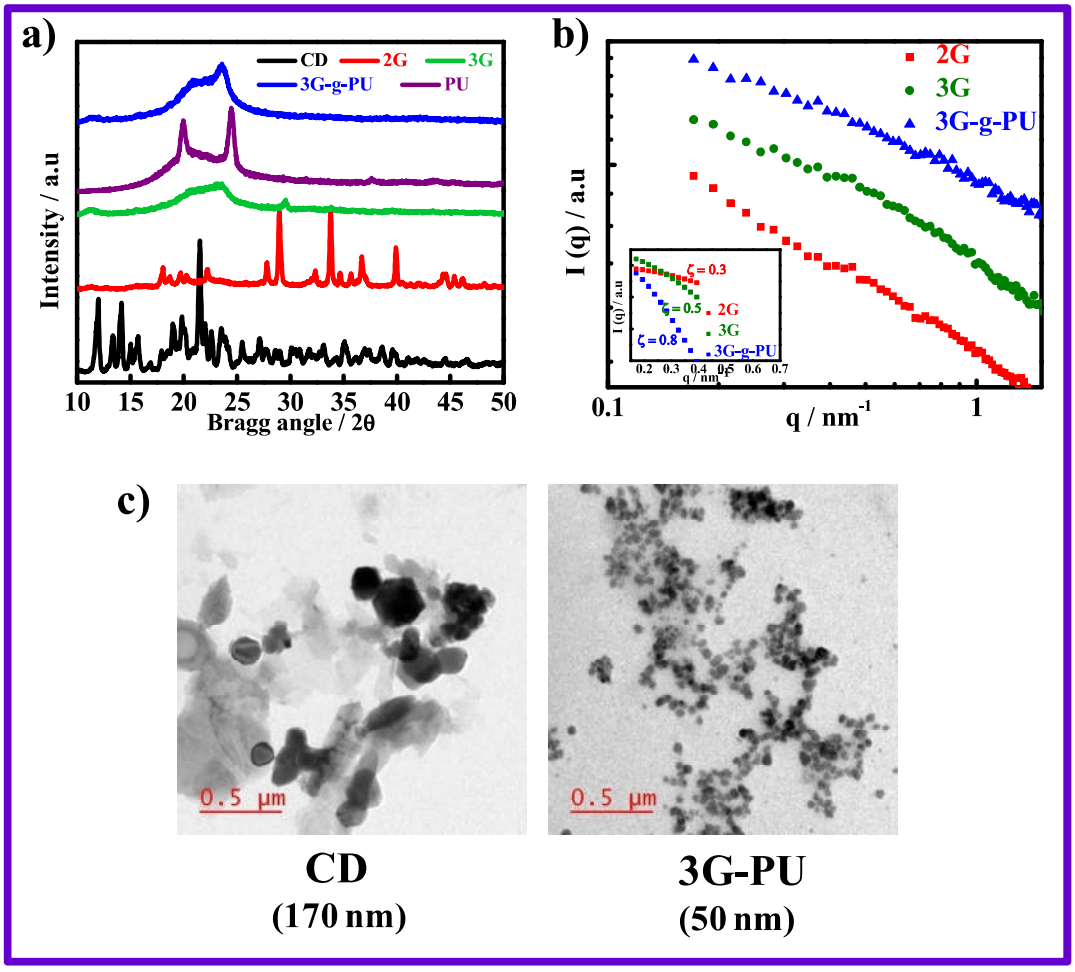

Figure 5.2: *Structural elucidation a) XRD pattern of all the systems showing reduced crystallinity with grafting. b) Small angle neutron scattering and its Debye-Buche fitting model in inset showing an increase in blob size with higher generations due to more interactions. c) TEM images of pure CD and 3G-PU.*

5.3 In-vitro drug release

The idea of designing generations and superstructure was to regulate the release of embedded guest molecules. Anti-cancerous paclitaxel is embedded in the superstructure and its release pattern in buffer solution is monitored as a function of time following the hydrophobic interactions with the designed generations of CD/superstructure. Steady and sustained release of drug is observed from 3G-g-PU (35% in 6 h) against burst release from pure CD (~100% in 6 h) and other generations (***Figure 5.3a***) presumably due to strong interaction between drug and vehicle and the barrier effect arising from the constraint cage-like matrix in 3G-g-PU. The location of drug molecule in generations and superstructure is schematically shown in (***Figure 5.3b)*** (left and middle cartoon) revealing the slow and sustained release of drug from 3G-g-PU as opposed to the quick release from CD/generations. The release kinetics of drug is better understood from the Korsmeyer-Peppas fittings (r^2~0.99), having exponent '*n*' values of 0.5, 0.6 and 0.7 for 2G-D, 3G-D, 3G-g-PU-D, respectively, indicating non-Fickian mode of release from the strongly interactive control system following the slow diffusion as the rate determining step out of processes like penetration of solvent into vehicles, dissolution of the drug and diffusion of the drug.[202] . Different release kinetics models like zero order , first order, Higuchi and Korsmeyer- Peppas are presented in ***Figure 5.4*** and ***table 5.1.*** These smaller particles having core shell structure with hydrophilic interior and hydrophobic exterior results in stronger hydrophobic interactions with drug molecules and ultimately leads to slower release as compared to CD as vehicle where burst release is observed. Wraping of hydrophobic polyurethane over the hydrophilic 3G helps in improving the relative overall hydrophobicity of the superstructure, as evident from contact angle of 70^o, 80^o and 90^o for

2G, 3G and 3G-g-PU, respectively, which in turn restrict the release of hydrophobic paclitaxel from the superstructure. Further, the strong interaction between drug and vehicle is understood from the calorimetric measurement, lower melting of drug loaded 3G-g-PU as compared to pristine 3G-g-PU (***Figure 5.3c)*** and intermediate peak position of $\pi \rightarrow \pi^*$ electronic transition of carbonyl ester of drug embedded 3G-g-PU with respect to pure drug and 3G-g-PU (***Figure 5.3d)***. Thus, the branched superstructure is found to be a novel delivery vehicle for sustained release of anti-cancer drug by creating a confined space along with suitable interactions.

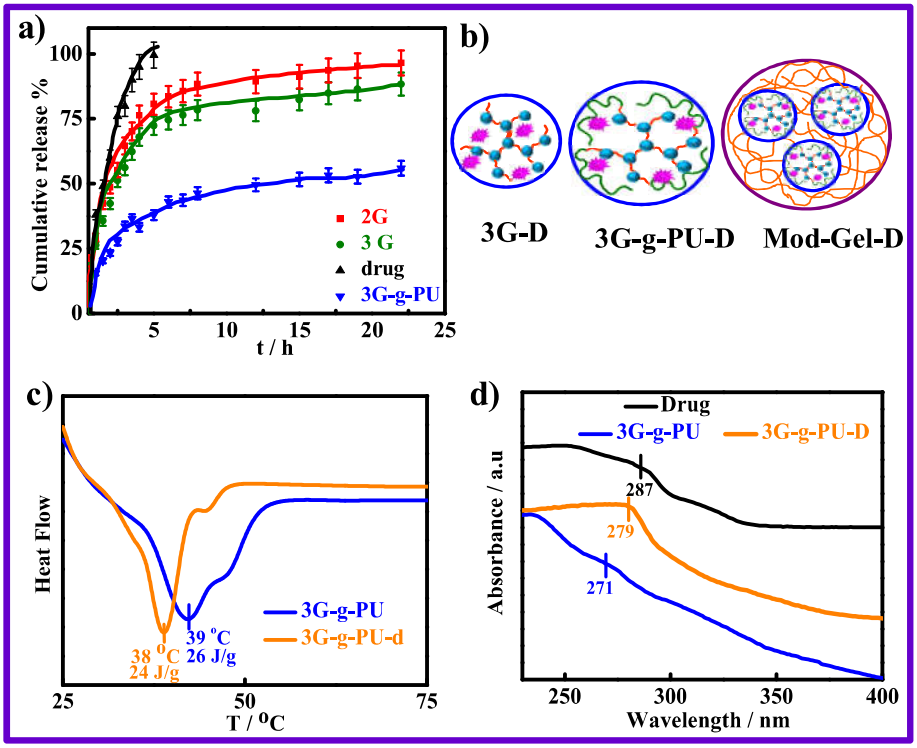

Figure 5.3: *a) Cumulative release of paclitaxel from CD, indicated generations showing sustained drug release profile from superstructure; b) Schematic model showing location of drug in various vehicles which cause sustained release from 3G-g-PU against fast release in 2G. Drug loaded 3G-g-PU is placed in MC hydrogel; c) DSC thermogrammes of with and without drug loaded 3G-g-PU showing the shift of melting peak and heat of fusion in presence of drug; d) UV-Vis spectra of indicated specimens showing polymer-drug interaction. Vertical lines indicate the respective peak positions.*

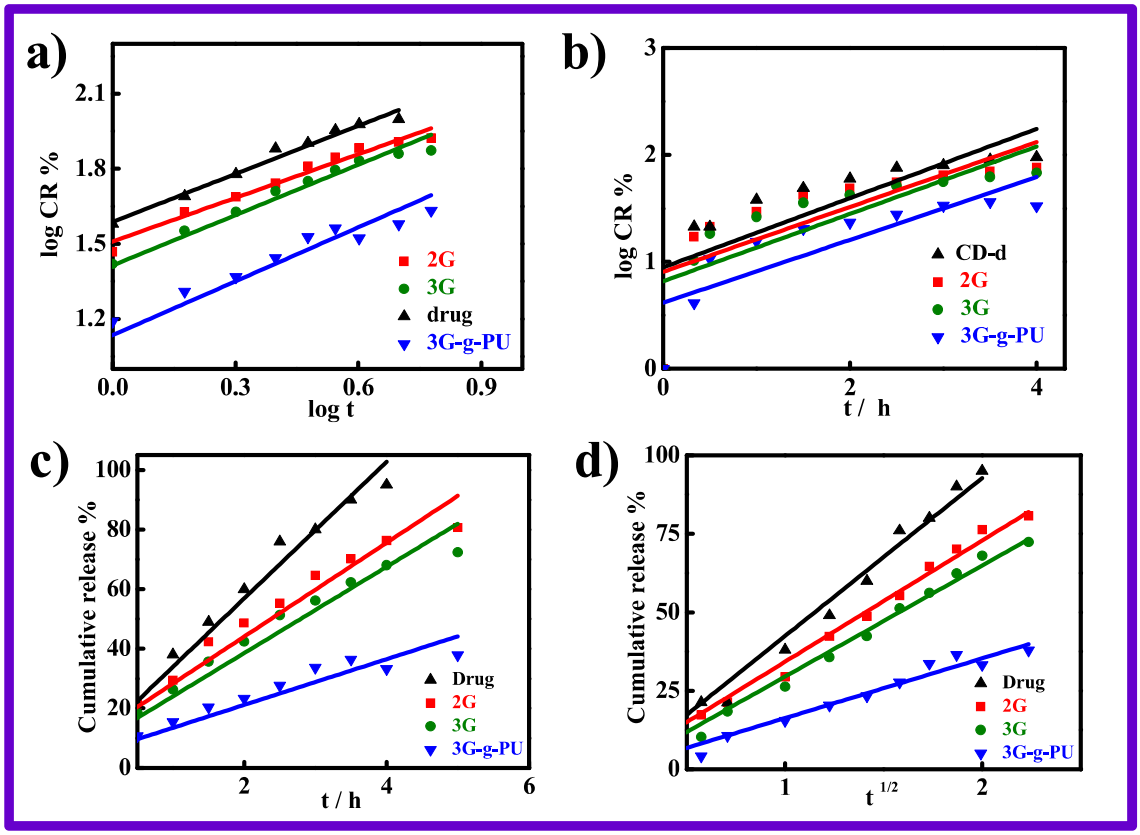

Figure 5.4: *Mathematical models for drug release kinetics a) Korsmeyer- Peppas model, b) First order model, c) Zero order model, d) Higuchi model.*

Table 5.1: *Release constant k, correlation coefficient (r), release exponent (n) calculated from various models for different genrations of CD*

Sample	First order		Zero order		Higuchi		Korsmeyer Peppas	
	k	r^2	k	r^2	k	r^2	n	r^2
drug	0.32±0.01	0.52	22.9.14±1.1	0.95	41.45±0.32	0.95	0.64	0.97
2G	0.300.02	0.52	15.70.12	0.94	13.8 ±1.6	0.98	0.58	0.98
3G	0.31±0.01	0.58	14.5±0.45	0.94	13.2±0.45	0.94	0.67	0.99
3G-g-PU	0.29± 0.01	0.63	9.640.45	0.84	19.0±0.04	0.93	0.71	0.98

5.4 In-vitro cytotoxicity

An effective drug delivery vehicle should be biocompatible in nature for its execution in biomedical fields. Biological responses of pure CD, 2G, 3G and 3G-g-PU are evaluated

through MTT assay using HeLa cell lines for five consecutive days and the superstructure is found to be better biocompatible as compared to the generations (***Figure 5.5a)*** as the wrapping (polyurethane) is well known biocompatible material and is widely used as implant in biomedical fields.[203] Further, the biocompatibility assessment is done through cell adhesion by taking phase contrast images of HeLa cells cultured over the materials which also support better cell adhesion on the bed of superstructure, as appeared from its well spreaded morphology (***Figure 5.5b)***. Thus, synthesized generations (2G, 3G) and 3G-g-PU have excellent biocompatibility and cell can grow very well on the surface of these materials and, thereby, found to be suitable carriers for drug delivery in real application. Now, it is pertinent to understand the efficacy of sustained release of drug from the superstructure towards cell killing with respect to CD or generations. Pure drug exhibits an increasing tendency of cell viability (less cell mortality) at longer time frame even though the cell killing efficiency is slightly higher in day 1 (***Figure 5.5c)***. Interestingly, the cell killing efficiency gradually increases for drug embedded 3G-g-PU and reach 75% in 5 days as opposed to meager 30% from pure drug (same concentration) in similar time. Pure drug is exposed to the cellular media immediately and can kill the cells to somewhat higher extent in day 1 while sustained release of drug from 3G-g-PU can able to kill the cells for very long period of time and attain very high cell killing efficiency using drug loaded superstructure. This is to mention that non availability of pure drug in longer time (already consumed in short time frame) cannot increase the cell killing rather cell proliferation occurs at longer time leading to higher cell viability or effective lower cell killing efficiency of pure drug. Drug concentration in the vehicle plays an improtant role and greater cell killing efficiency is observed in higher drug (***Figure 5.5c & d)***.

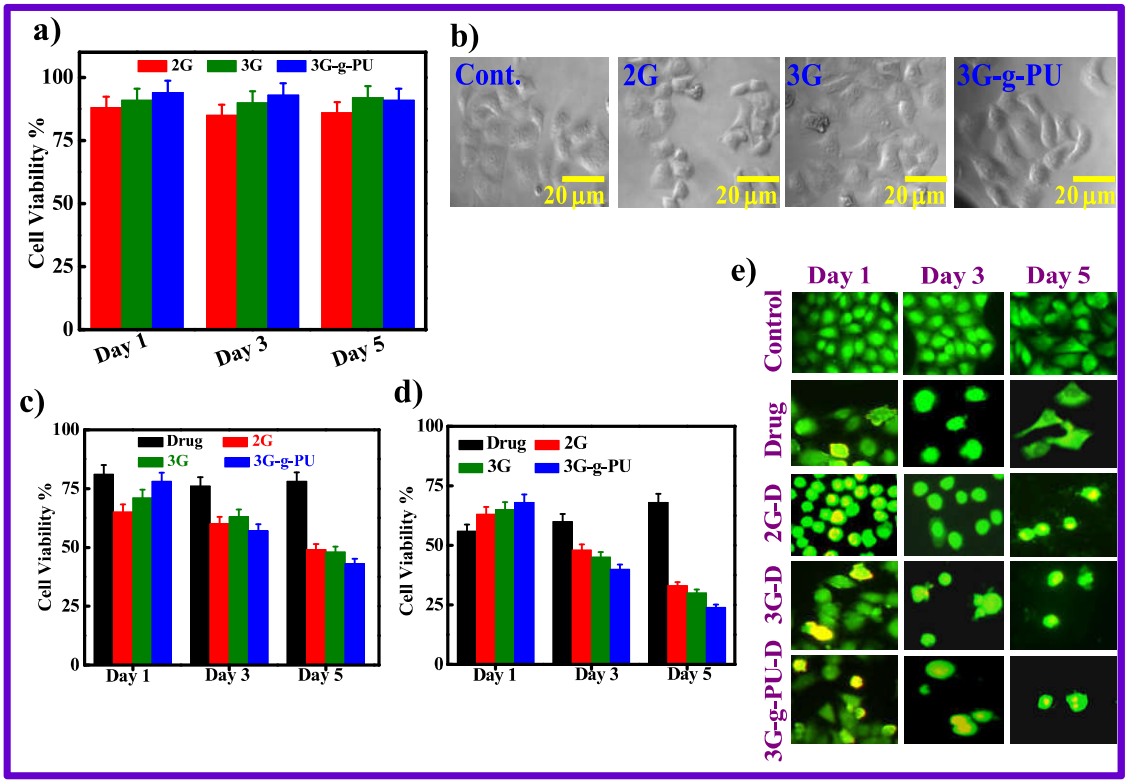

***Figure 5.5**: a) Cell viability of indicated samples at indicated time interval through MTT assay measurement; b) Phase contrast images of cells grown on different indicated sample surfaces (cell adhesion); In-vitro cytotoxicity (cell killing) by pure drug and drug loaded 3G-g-PU against HeLa cells at different doses c) at 20 µg/ml, d) at 200 µg/ml as a function of time; and e) Fluorescence images after AO/EB staining of cells treated with pure drug and drug loaded indicated specimens showing differential cell density.*

For better understanding the mechanism of cell killing or induced apoptosis by drug loaded vehicles/pure drug, the cells are stained with acridine orange (AO) and ethidium dibromide (EtBr) and are observed under fluorescent microscope. This dual staining procedure helps to differentiate between normal and apoptotic cells on the permeability of cell membrane. AO is permeable to cell membrane and binds with DNA and displays green fluorescence, while EtBr is primarily taken up by the apoptotic cells and stains the nuclei with red fluorescence.[204] AO/EtBr stained cells appear as orange yellow signifying apoptosis or the beginning of necrosis providing red colour fluorescence as their membrane integrity is

lost while the normal healthy cells fluoresce in green color.[205] Very less cell density and mostly apoptotic nature is observed in cell line treated with drug loaded 3G-g-PU while healthy cells are observed in day 5 in cell line treated with pure drug **(*Figure 5.5e)*** commensurating with the global observation through MTT assay *cf.* **(*Figure 5.5d)***. Thus, the effect of sustained release is very much prominent in cellular studies and the efficacy of the superstructure as the superior delivery vehicle is demonstrated with plausible mechanism.

5.5 In Vivo efficiency

Finally, the effect of sustained drug delivery of 3G-g-PU on mouse bearing melanoma tumor, induced using B16-F10 melanoma cell line, is evaluated by treating with the patch consisting of drug embedded 3G-g-PU over the tumor site. This patch considerably suppress the tumor growth as compared to control (***Figure 5.6a)*** while no healing/shrinkage of tumor is observed like the reported systems using 5-Fu loaded MCL (a diblock copolymer of MPEG-b-(PCL-ran-PLLA) as an injectable hydrogel which could only inhibit the tumor growth by 3 times of the initial tumor against 60 times increase in that of control after 18 days.**[206]** In another report, MPEG-PCL based diblock copolymer loaded with paclitaxel as intra tumoral drug depot inhibited tumor growth rate (mm^3/day) 20.6 against 96.4 in control (without any treatment).**[207]** The interfacial constraint (insufficient contact) between patch and tumor surface lead to inadequate solid state diffusion of drug which results in lesser drug available for tumor healing. In order to circumvent the drawback, the idea of injectable gel comes into picture with the understanding that the gel will be in contact just below the tumor site at subcutaneous

position. Methyl cellulose is the right option considering its biocompatibility**[208]** and the formation of injectable gel using appropriate concentration.

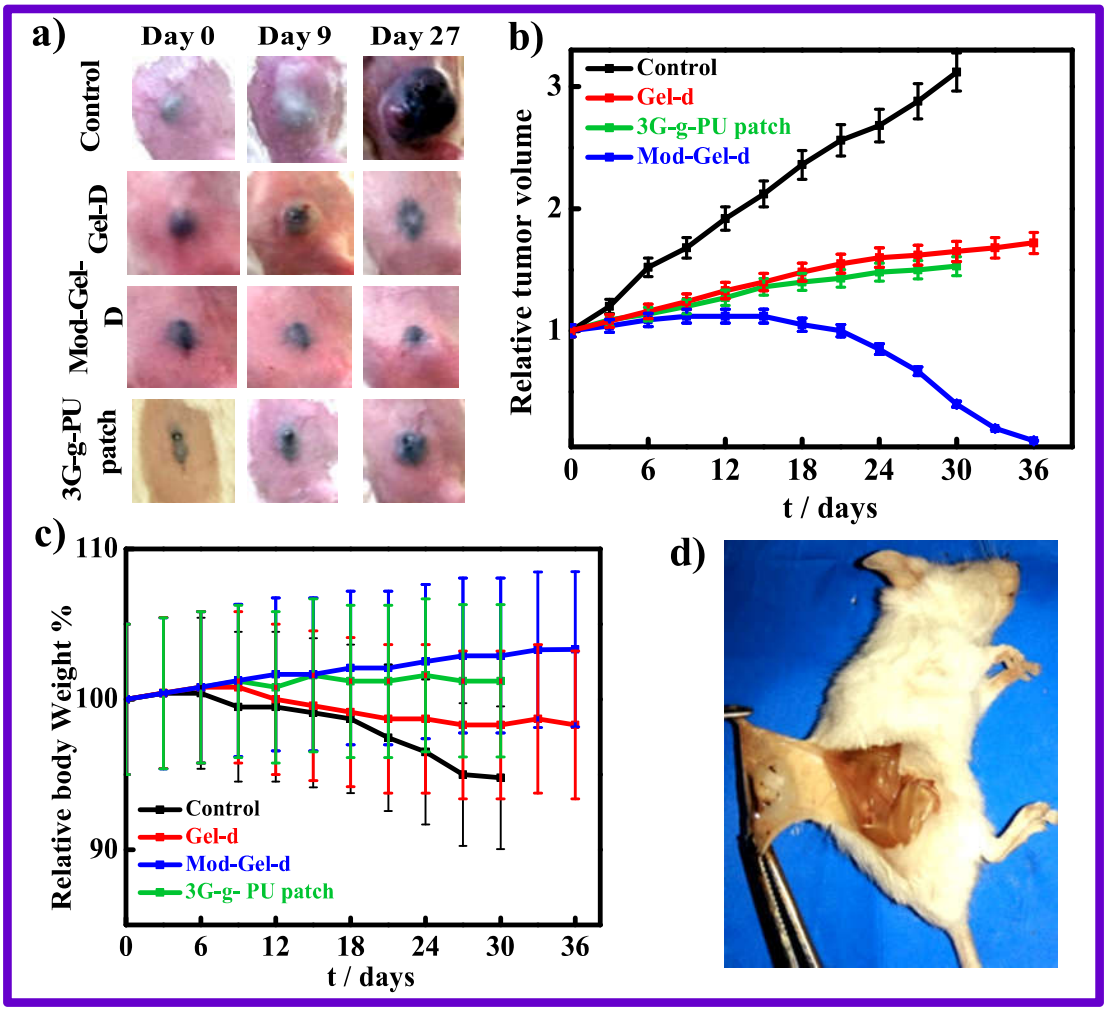

Figure 5.6: *a) Images of mice at initial day and after treatment with Mod-Gel-D and Gel-D indicated systems for varying time interval. Black spots indicate the cells/area containing melanin while tumor size is measured from the swollen area of respective mice; b) Relative changes in tumor volume with time treated with indicated Gels and patch; c) Relative changes in body weigh with time treated with indicated Gels and patch; d) Mice showing the formation of hydrogel after subcutaneous injection.*

Drug embedded superstructure (3G-g-PU-d) is placed in gel (through solution route) following the schematic shown in (***Figure 5.3b)*** and is termed as '*Mod Gel-D*' while pure

drug embedded in methyl cellulose gel is considered as control and designate as '*Gel-D*'. The advantage of this system is that the whole system becomes injectable gel and one can place drug embedded gel just below the tumor site for constant drug diffusion and body fluid help in this process by lowering the viscosity. The anti tumor efficacy of *Mod-Gel-D* is investigated using B16-F10 melanoma model in albino mice and the treatment is initiated when the tumor volume reached approximately 20 ± 3 mm^3. As usual, the tumor volume keep on increasing with time in control (no treatment) while interestingly, the tumor volume decrease with time in mice group treated with *Mod-Gel-D*. (***Figure 5.6b)*** quantifies the relative tumor volume as a function of time clearly indicating the complete healing of tumor using *Mod-Gel-D* treated mice while similar amount of drug embedded in MC gel (*Gel-D*) just suppress the tumor growth, as compared to control, but no healing or shrinkage is observed. The sustained release of drug from the superstructure (3G-g-PU) achieve the therapeutic dose over a longer period of time (slow but steady release as evident from (***Figure 5.3a***) just below the tumor tissue through MC gel. On the other hand, fast release of drug from *Gel-D* exceeds the therapeutic dose in very short period of time and thereby, diffuses into the blood stream. As a result, tumor tissues do not get the required drug concentration in longer period for *Gel-D* treated mice affecting the tumor growth suppression. Further, MC has another advantage of specifically bind to melanin present in melanocytes as evident from the accumulation of *Mod-Gel-D* or *Gel-D* exclusively at the tumor site (***Figure 5.6a)***

However, slow but steady release, so-called sustained release from Mod-Gel-D using superstructure shrinks the tumor completely while most of the drug from Gel-D enter into blood stream, from its fast release kinetics, and expected to affect the other body

organs/toxicity unlike Mod-Gel-D. Conversely, the food intake of mice treated with Gel-D and pure drug decrease considerably which is consistent with the body weight index while there is gradual increase of body mass of the mice treated with Mod-Gel-D.

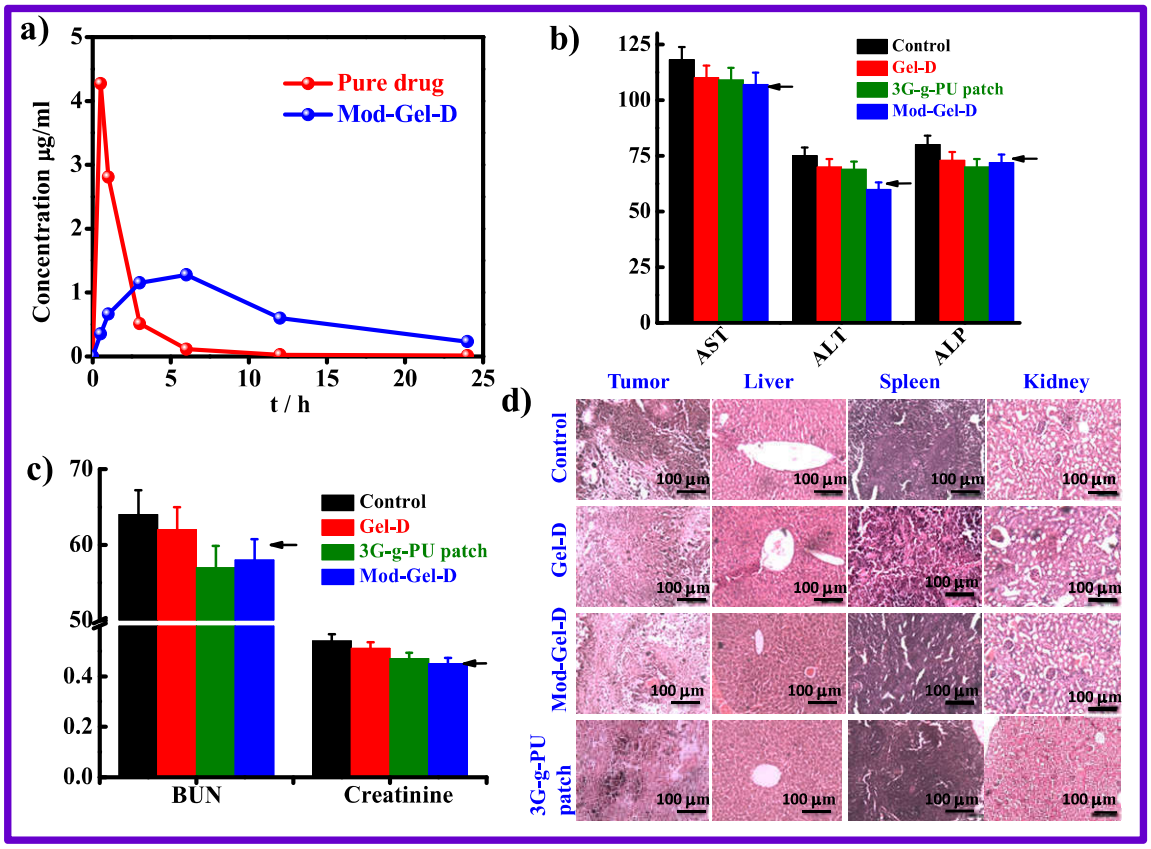

Figure 5.7: *a) Plasma drug concentration versus time profile for pure drug and Mod-gel-D treated albino mice after intravenous administration at 5 mg/kg body weight and an equivalent amount of drug in Mod-Gel. Biochemical parameters, b) Hepatic function test including AST, ALT, and ALP c) Renal function namely BUN and Creatinine of the mice treated with Gels. Corresponding values of healthy mice are indicated by the arrows; d) Histopathological analysis of organs like liver, kidney, spleen and tumor dissected from mice after 30 days of treatment. Results are presented in mean value ±SD, n=5. ***p<0.001, **p<0.05 and *p<0.01.*

Toxicity of drug is one of the important and leading factors which cause the failure of cancer therapy in most cases.[209] The purpose of this study is to investigate whether the

Mod-Gel-d can successfully deliver the drug in a sustained manner by maintaining the drug concentration in blood plasma within the therapeutic level over a prolonged time and thereby can reduce the adverse side effects caused by the burst release of pure drug. For comparing the in-vivo performance of pure drug and Mod-Gel-D, the concentration of drug in blood stream is measured after an intravenous dose of 5 mg/kg of paclitaxel. (***Figure 5.7a)*** shows the concentration-time curve of paclitaxel determined after the different time interval after single administration. The plasma concentration of paclitaxel above a threshold value of 0.1 μM (equivalent to 85.3 ng/ml) is pharmacologically active.[210] It is observed that after the administration of both the systems, generally a decline in paclitaxel concentration has been observed with initial burst release (C_{max} 4.2 μg/ml at 0.5 h) followed by its quick elimination. While the paclitaxel in Mod-Gel-D exhibited sustained release with minimal initial burst release and attain a higher concentration C_{max} of 1.2 μg/ml in 6 h demonstrating that paclitaxel in pure form is rapidly cleared from the circulatory system (within 3 h of administration) while the paclitaxel loaded in Mod-Gel is retained for a longer time in blood stream. These results indicate the efficacy of sustained drug release from Mod-Gel-D by increasing the residential time and slower drug elimination. However, the embedment of 3G-g-PU within MC gel further causes sustained release which affects the circulation time and is known to affect the uptake by the reticuloendothelial system.

Biochemical analysis including liver function tests has been carried out to examine the hepatic dysfunctions caused by the drug uptake. Liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the critical parameters for assessing the proper liver functioning in albino mice after treatment. Considerably

increased level in the activity of ALT and AST is obvious in mice group treated with Gel-D (ALT ~ 70 U/L and AST ~ 110 U/L) after 30 days of treatment along with significant increase in control group while the mice group treated with Mod-Gel-D exhibit normal level of the enzymes (indicated by the respective arrows) **(*Figure 5.7b*)** implying healthy mice whose tumors have been healed/shrink completely.

This is worthy to mention that ALT and AST in mice treated with drug-loaded 3G-g-PU patch is almost similar to Mod Gel-D due to slow release of drug from patch Figure 5.7b.[211] Similarly, renal function tests mainly serum urea and creatinine levels are also studied to monitor the renal toxicity in all the mice (treated with Gel-D, Mod-Gel-D and patch of 3G-g-PU-D). Urea and creatinine levels have risen in mice treated with Gel-D (~ 20 and 11%) after the complete treatment time of 30 days) while almost normal level is observed in mice treated with Mod-Gel-D or patch. This is to mention that the urea and creatinine levels in control groups are almost similar to Gel-D treated mice (***Figure 5.7c)***. As a consequence, liver and kidney are severely affected by the administration of pure drug[212], while the significant improvement has occurred in the cases using drug embedded superstructure through the injectable gel.

Histological analysis of internal organs for understanding the effect of drug on vital body organs mainly liver, kidney and spleen along with tumor tissue are also performed to estimate the efficacy of the vehicle towards healthy tissues or vital body organs. The tumor treated with Mod-Gel-D exhibits severe cell death showing sufficiently higher necrotic spots with extensive nuclear shrinkages and fragmentation as opposed to high and moderate cell density of control and Gel-D treated mice group arising from their respective cell killing efficiency ***(Figure 5.7d).*** These therapeutic effects are likely due to sustained

drug release from Mod-Gel-D maintaining the concentration of drug at the local tumor regions. At the same time, bioavailability of drug is preserved due to sustained release kinetics causing inhibition of melanoma tumor growth. Liver histogram of mice in Mod-Gel-D treated group displays normal architecture of hepatocytes while mice administered with Gel-D and control exhibit significant damage showing inflammation in portal tract and deformation in size and shape of hepatocytes. Degeneration of epithelial cloudy cells further support liver damage in control and Gel-D treated groups. However, no such acute toxicity is observed in the liver of mice treated with Mod-Gel-D. Kidney of mice treated with Gel-D shows slight focal tubular injury and the kidney health is preserved in rest other groups. Spleens in all the systems are found to be normal and display no leisons of damage except slight damage in Gel-D treated mice group where slight inflammation is observed. However, pure drug is found to damage the other healthy tissues/organ, as usual for conventional therapy of cancer treatment arising from its fast release / exposure at short time which exceed the therapeutic window, while sustained release from the developed vehicle do not affect the other body organs as the drug concentration remain within the therapeutic window for longer period of time and kill the tumor cells efficiently.

5.6 Conclusion

Intricate architecture of CD (3G) has been designed followed by its grafting with hydrophobic polyurethane at the periphery to control the hydrophilic hydrophobic balance to regulate the release of hydrophobic anti-cancerous drug (paclitaxel). The efficacy of the vehicle is examined for its sustained release which follows non-Fickian kinetics, a diffusion controlled process. The developed material is biocompatible while the drug embedded superstructure exhibit very high cell killing efficiency (75%) as compared to

pure drug in similar concentration and time. The greater interaction, between drug and superstructure revealed through calorimetric and spectroscopic studies, along with hydrophilic hydrophobic balance of the superstructure are responsible for its controlled release capability. The embedment of drug loaded superstructure in injectable hydrogel makes this vehicle possible to place it just below the tumor site for best utility through effective drug delivery at local site. In-vivo studies using albino mice clearly demonstrate the efficacy of complete healing of tumor in one month time without any side effect, as usually observed in conventional chemotherapy. Thus, biocompatible and cell killing property of drug loaded superstructure acts as versatile drug delivery carrier for subcutaneous delivery of therapeutic agents particularly for tumor treatment and shows its suitability for cancer treatment in future without any side effect.